

**NOAA
FISHERIES**

Northwest
Fisheries
Science Center

9.2 What is epigenetics and why could it be relevant to recovery and management of protected resources?

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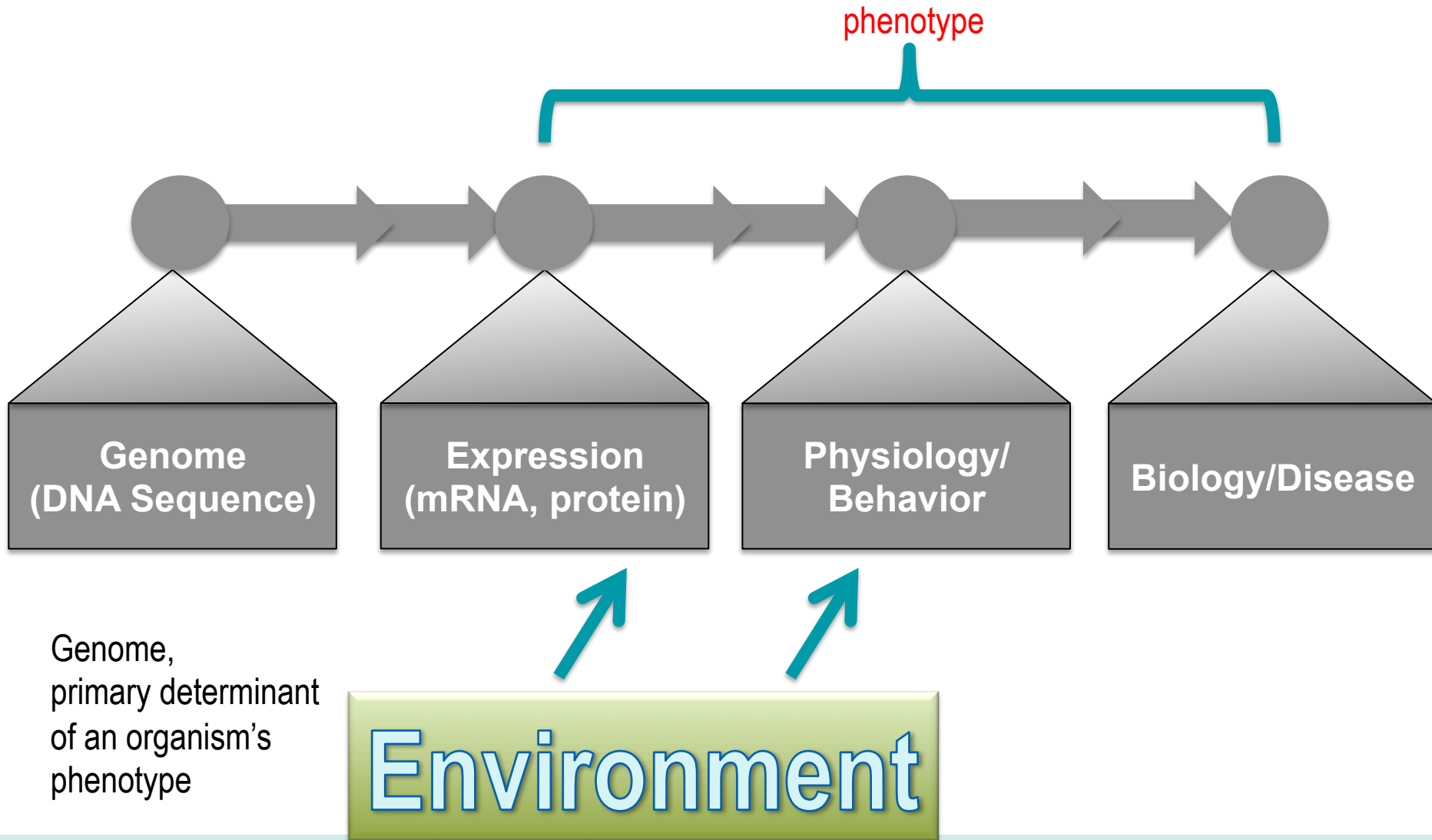
Funded by Bonneville Power Administration
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May 6, 2015

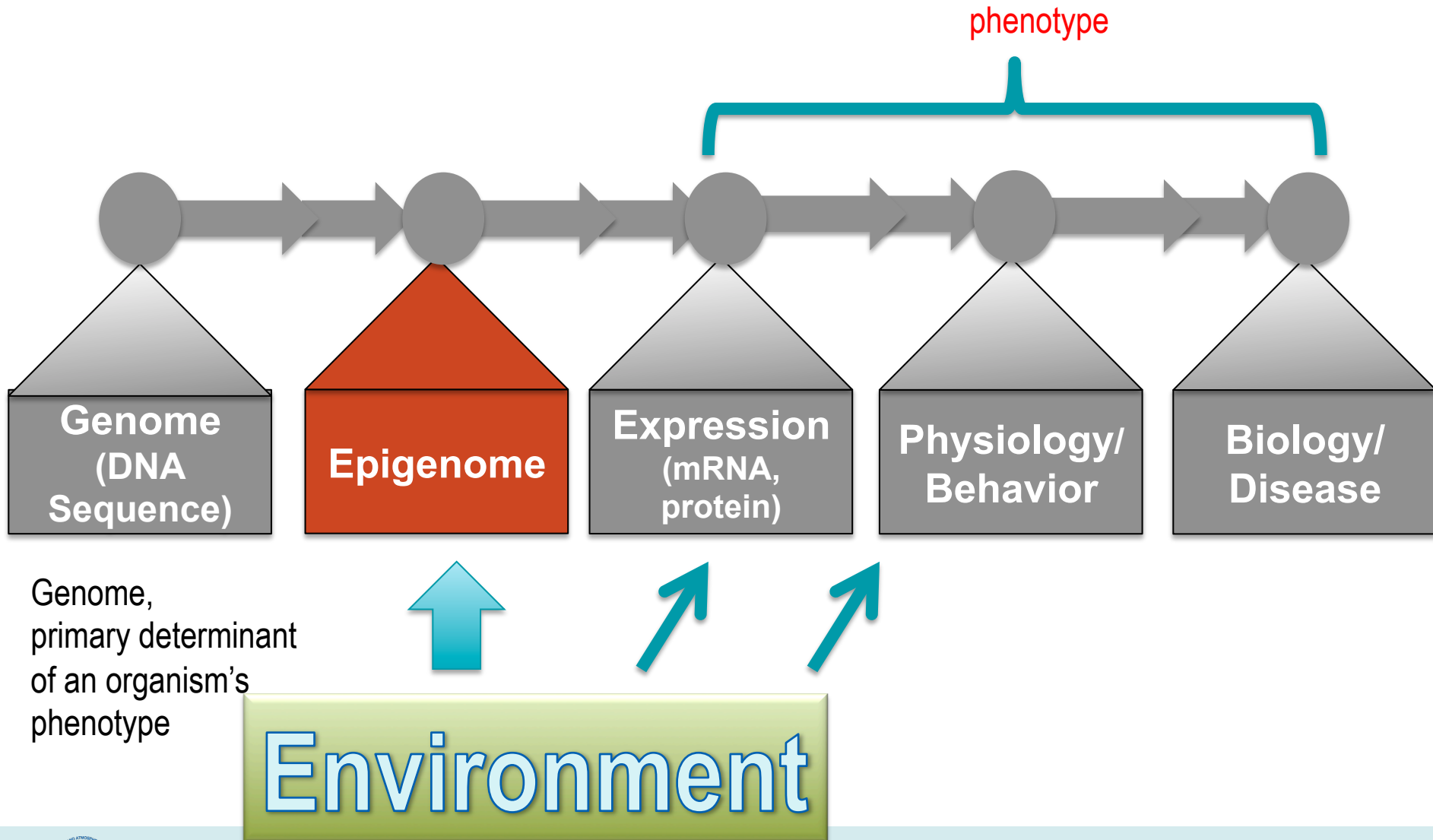
Problem

- Hatchery programs may reduce the “fitness” of salmon and steelhead spawning in the nature
- Plausible mechanisms:
 - Hatchery-induced selection that causes rapid genetic change
 - Environmentally-induced changes in juveniles that alter:
 - Adult phenotype and reduce breeding success
 - Non-genetic factors in germ cells that affect offspring survival
 - Both

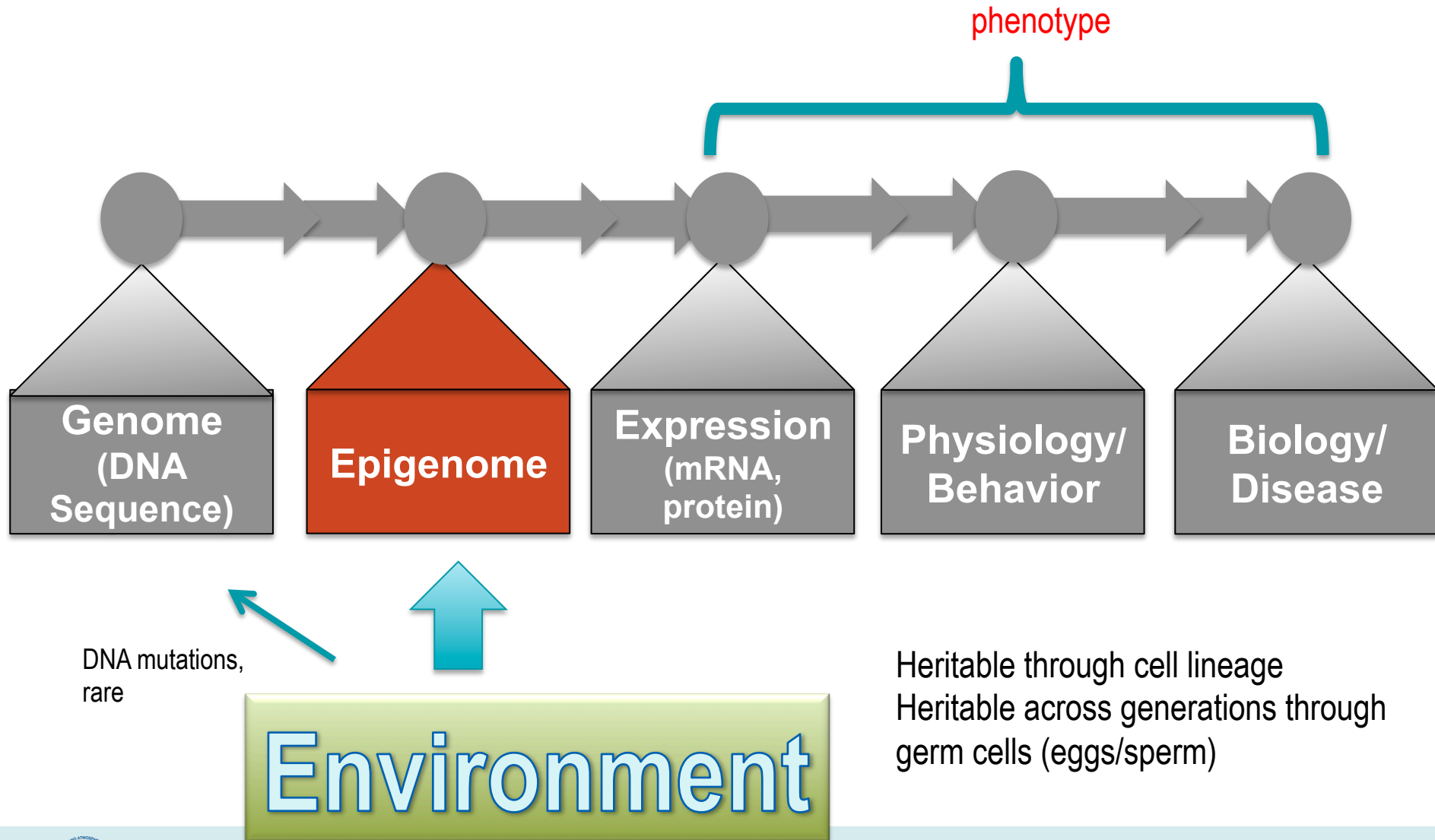
Genetic x Environment → Phenotype



Genetic x Environment → Phenotype

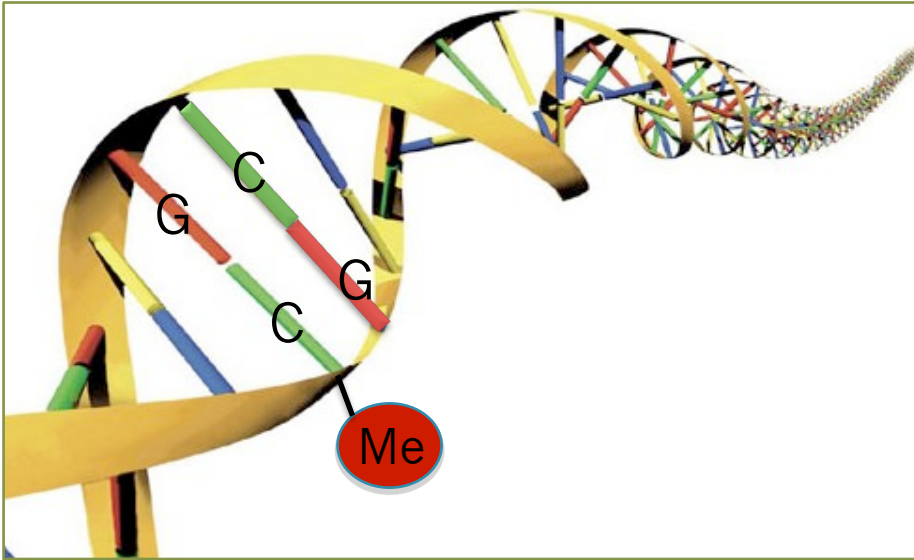


Heritable changes that affect phenotype



DNA Methylation

- Most well understood epigenetic mechanism is **DNA methylation**
 - Methylation of CpG occurs in most plants and animals
 - Typically* associated with gene silencing if in promotor region of gene
 - Methylation in gene bodies associated with enhanced transcription, formation of splice variants



Examples of change in DNA methylation associated with phenotypic change

- Temperature- sex change
- Aging- twins
- Domestication-stress response
- Behavior- maternal care, anxiety
- Nutrition/Toxins-coat color, obesity



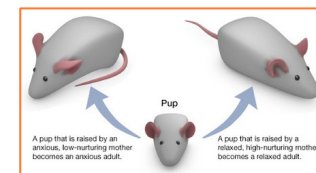
PLoS Genet 7: e1002447



PNAS (2005)102:10604-10609



BMC Genomics (2012) 13: 59



<http://learn.genetics.utah.edu/content/epigenetics/rats/>

Nature Neuroscience (2004) 7:847–854



Environ. Health Perspect (2006)114:567–572

Implications for Evolution

- Genome changes slowly, through random mutation and natural selection on the phenotype. Takes many generations for genetic trait to be common in a population
- Epigenome changes in response to signals from the environment and can happen in many individuals at once. Some MAY be passed on to future generations. Through epigenetic inheritance, experiences of parents may pass to future generations.
- Epigenome is flexible, allows organism to adjust gene expression according to environment without changing DNA code
- Epigenetic changes can lead to genetic changes

Why could epigenetics be relevant to understanding loss of fitness of hatchery fish, or rapid adaptation and evolution?

Hatchery rearing environment differs from nature and is manipulated to meet production goals

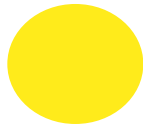
- Embryo incubation temperature- alters seasonal timing of emergence & ponding of fry
- Energy content of diet higher than natural diet
- Seasonal pattern of juvenile growth different than in nature
- Rearing density higher (can affect stress)
- Lack of predators

Potential factors that could induce heritable changes in the epigenome

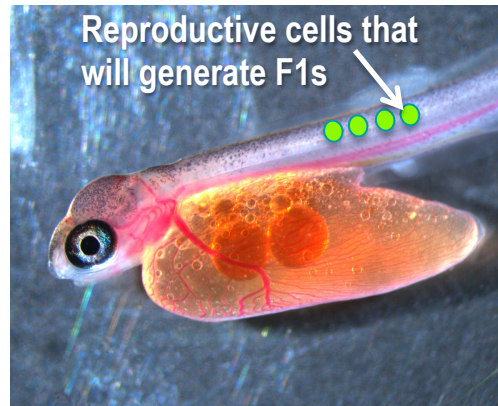
photoperiod



temperature



*Olfactory
cues*



Hormones in the yolk

*Water chemistry
(pH, organics,
toxins, O₂)*

*Nutrients
(yolk and exogenous food)*

Overarching Questions

Do hatcheries, changes in habitat, and long term climate change contribute to inherited epigenetic programming that influences life history, survival and reproduction of salmonids?

Are there epigenetic signatures in the same parts of the genome across populations and can we detect them through screening?

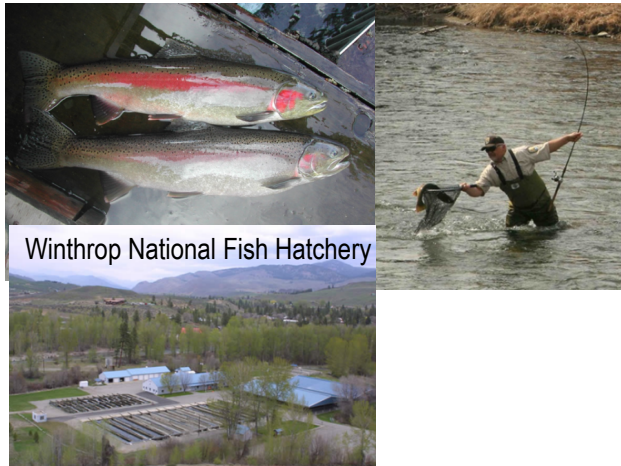
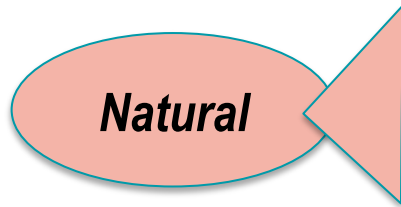
Research Questions

Are there discernable epigenetic and/or genetic differences between hatchery and natural origin steelhead that could be passed to subsequent generations?

If so, does it differ between hatchery rearing protocols (yearling vs two-year old smolt production)?

Experimental Design:

Compare genetic and epigenetic variation in natural and hatchery origin Methow River steelhead



- Returning Methow River steelhead adults collected by angling
- Collect fin clip, blood, gametes

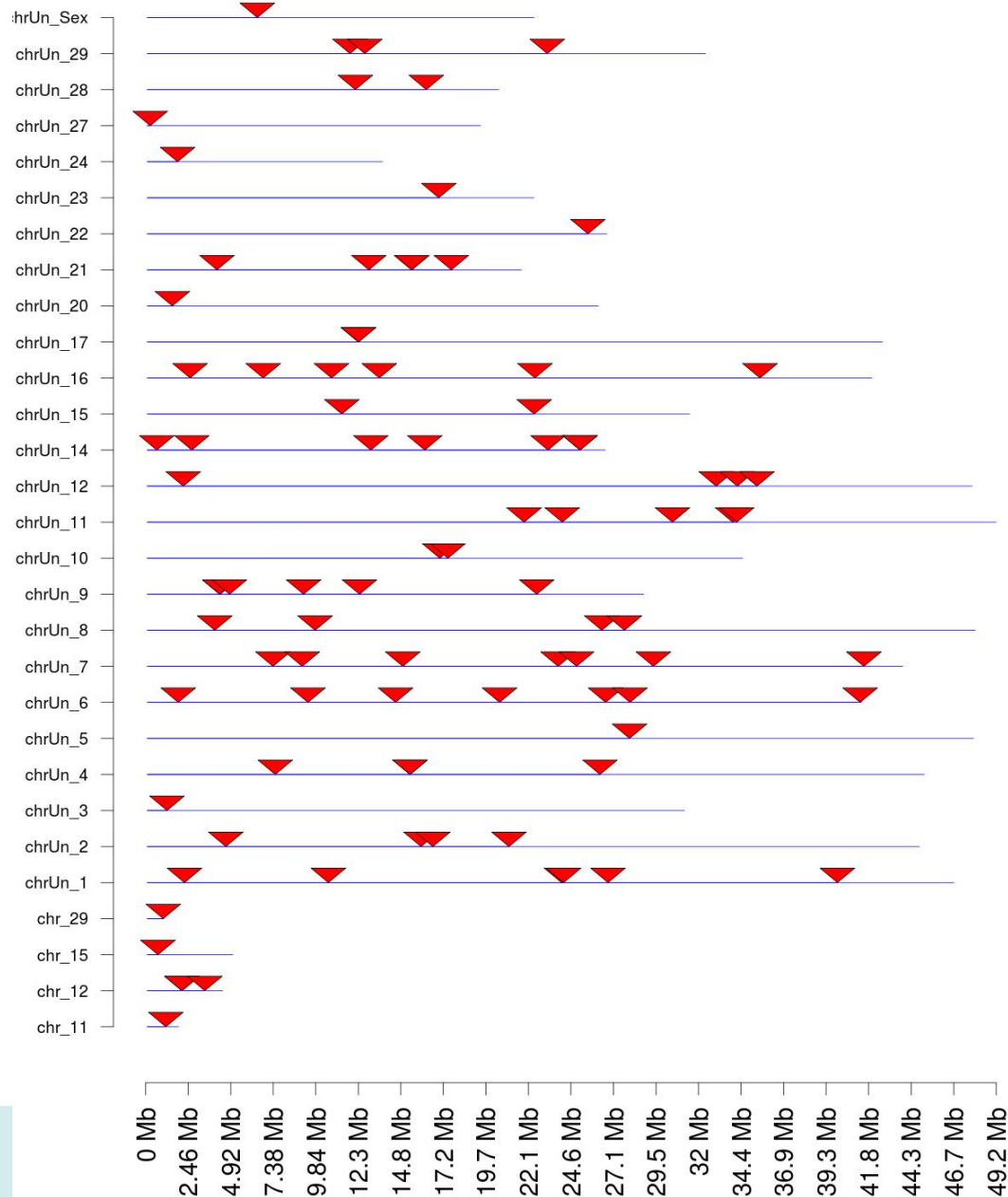
No discrimination between natural & hatchery origin fish

- **Epigenetic analysis:** Genome wide DNA methylation analysis in sperm, RRBS

Preliminary Results: Epigenetics

- 74 differentially methylated CpG Regions (DMRs) in sperm DNA
 - 45 hypermethylated in hatchery fish
 - 29 hypomethylated in hatchery fish
- Almost half of the DMRs are located within 5kb of genes (potential gene regulatory regions), one third within gene bodies
- Differences could be due to early rearing environment, but cannot rule out age.
- Currently analyzing additional samples- comparing two hatchery rearing methods (yearling vs 2-year old smolt); test for age and rearing environment effects

Natural versus Hatchery Methow River Steelhead



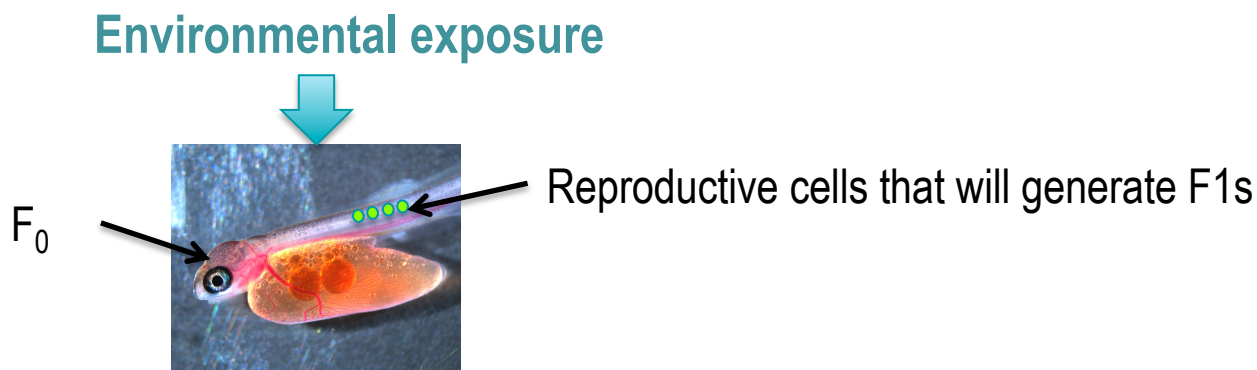
Chromosomal locations of differentially methylated regions of sperm DNA in natural vs hatchery origin (S1) Methow River steelhead

What about transgenerational effects?

Establishing epigenetic differences in sperm DNA from natural and hatchery origin fish is compelling, but does not prove these are heritable.

Challenges of Proving Transgenerational Epigenetic Inheritance

- Must rule out possibility of genetic change
- Must show that the epigenetic effect can pass through several generations to rule out possibility of direct exposure



New Study- Transgenerational Epigenetic Inheritance

Hypothesis- Early Rearing Environment Alters Epigenetic Programming of Steelhead Germ Line

20 families split across two rearing environments

Hatchery



Artificial Stream



Study Design:

**Collect gametes and eyed embryos from natural origin
Methow River adults (20 families)**

YEAR 1

Hatchery

- Heath trays & tanks
- Artificial Diet
- Delayed embryo development
- Reduced incubation temperature

Artificial Stream

- Buried in gravel
- Natural Diet
- Ambient temperature

YEARS 2-4

- Rear in common garden tanks
- Collect Sperm & RBCs from maturing males
- Compare DNA methylation patterns across treatments, families, and offspring relative to fathers

Phenotype of Steelhead from Hatchery vs Artificial Stream Environment: 8 months of rearing

Hatchery



Artificial Stream



Bar = 100 mm

Next steps: 1-2 more years of rearing needed to obtain maturing males for sperm DNA methylation analysis. Test for rearing environment effects. Compare paternal and offspring methylation patterns.

Summary

- Assembled a strong interdisciplinary team to investigate role of epigenetics in phenotypic plasticity, adaptation and evolution in *O. mykiss*
 - *Expertise in molecular and quantitative genetics, functional genomics, fish physiology, behavioral ecology, and bioinformatics*
 - *Facilities for lab studies, hatchery rearing, computer cluster for bioinformatics*
 - Partnerships with USFWS, UW, WSU, and Institut National de la Recherche Agronomique (INRA)
- Established laboratory methodologies for analyzing DNA methylation and bioinformatic pipelines to analyze data
- Found significant differences in methylation of sperm DNA due to hatchery rearing or age. Suggest potential for heritable epigenetic effects of early rearing environment.
- Studies initiated to test for transgenerational epigenetic inheritance

What are the challenges?

- Bioinformatics challenging because of genome duplications in salmon and steelhead
- Data analyses in other Pacific salmon species without genome sequence difficult
- Linking specific differences in epigenetic programming to functional differences, phenotypes
- Costs of the analysis
- Long-term funding for transgenerational studies in species with 2-4 year generation times

Where could this research go?

- Reforming hatchery rearing practices (rearing densities, diet, feeding regimes, early rearing protocols) to reduce impacts
- Development of epigenetic signatures of wild and hatchery fish of same stock that cannot be discriminated with genetic analysis
- Understanding scope of adaptation to extreme changes in environment
- Numerous applications to habitat restoration and toxicological research
- If epigenetic changes occur in hatchery, what happens after one or more generations in the wild?

Acknowledgements

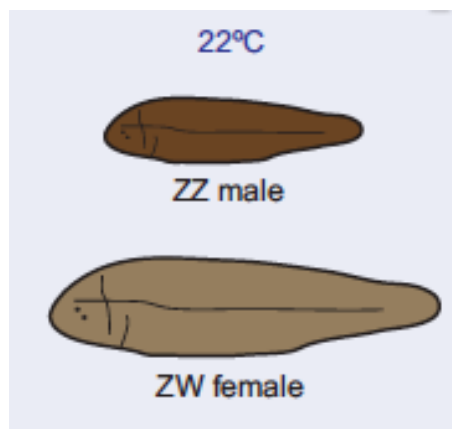
- Funding from Bonneville Power Administration & NOAA Fisheries
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- Chris Pasley, USFWS-WNFH
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- Numerous volunteers that caught fish
- Mike Skinner, Washington State University
- Craig Busack, WRO NOAA Fisheries

Questions?



Story of the half-smooth tongue sole.....

Genetic Sex Determination
@ 22 °C
during early development



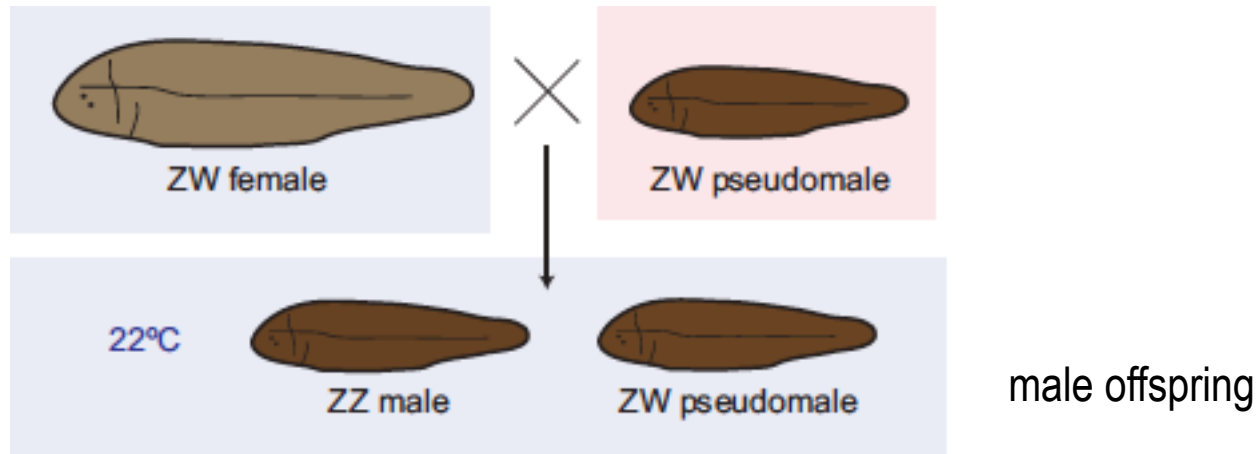
Genetic females sex reversed
@ 28 °C
during early development



Chen et al. *Nature Genetics* **46**, 253–260 (2014)
Shao et al. *Genome Research* **24**: 604-615 (2014)

Story of the half-smooth tongue sole.....

Breeding genetic female with sex reversed male
results in 100% males



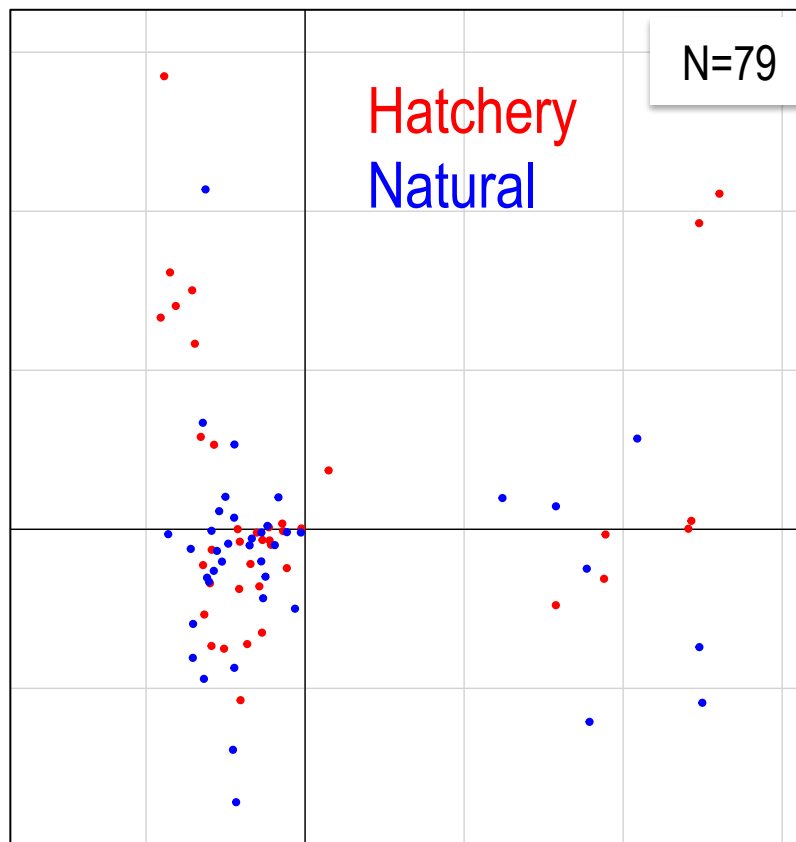
Environmentally-induced change in phenotype was
passed on to offspring with no genetic change.

How?

Chen et al. *Nature Genetics* **46**, 253–260 (2014)
Shao et al. *Genome Res.* **24**: 604-615 (2014)

Results: Genetics

Genetic Analysis of Natural and Hatchery Origin Methow River Steelhead: Genome Wide Assessment of SNPs

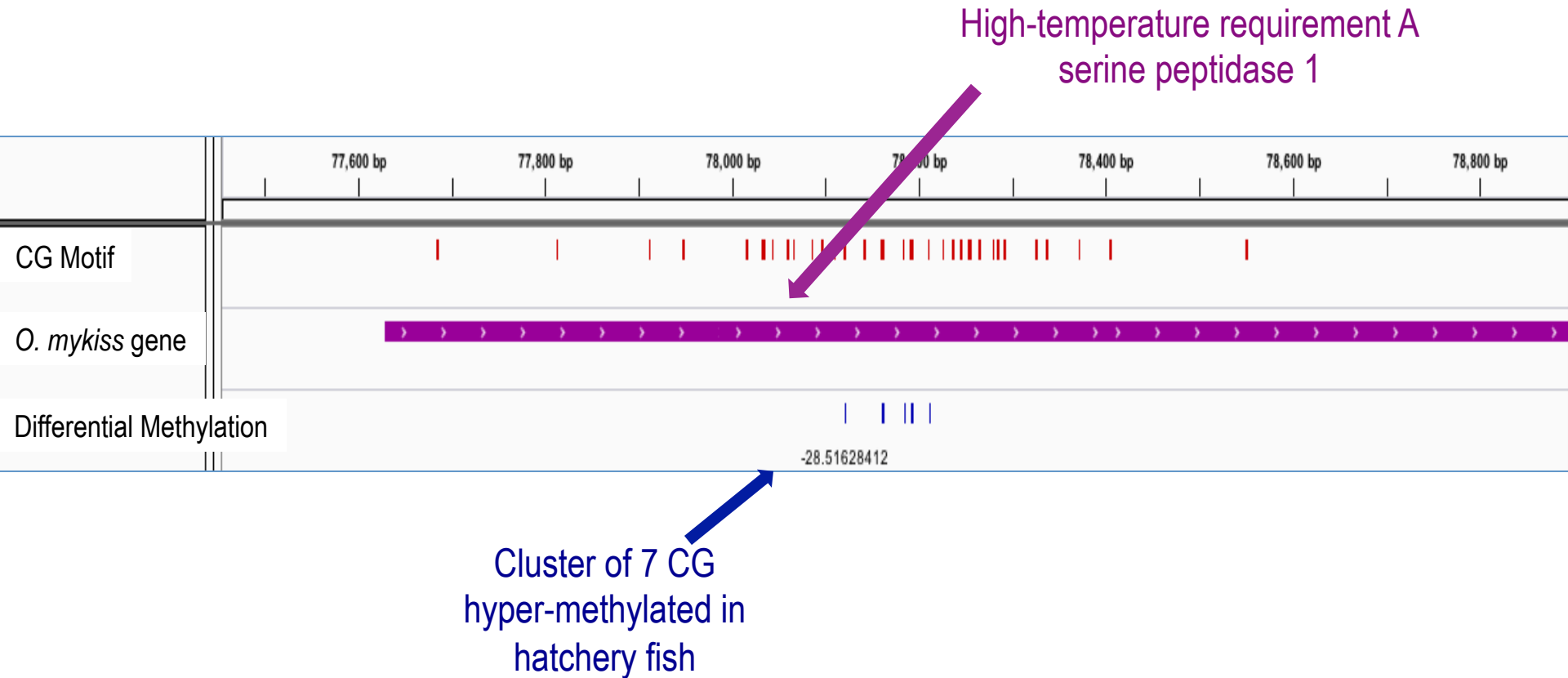


Preliminary results:

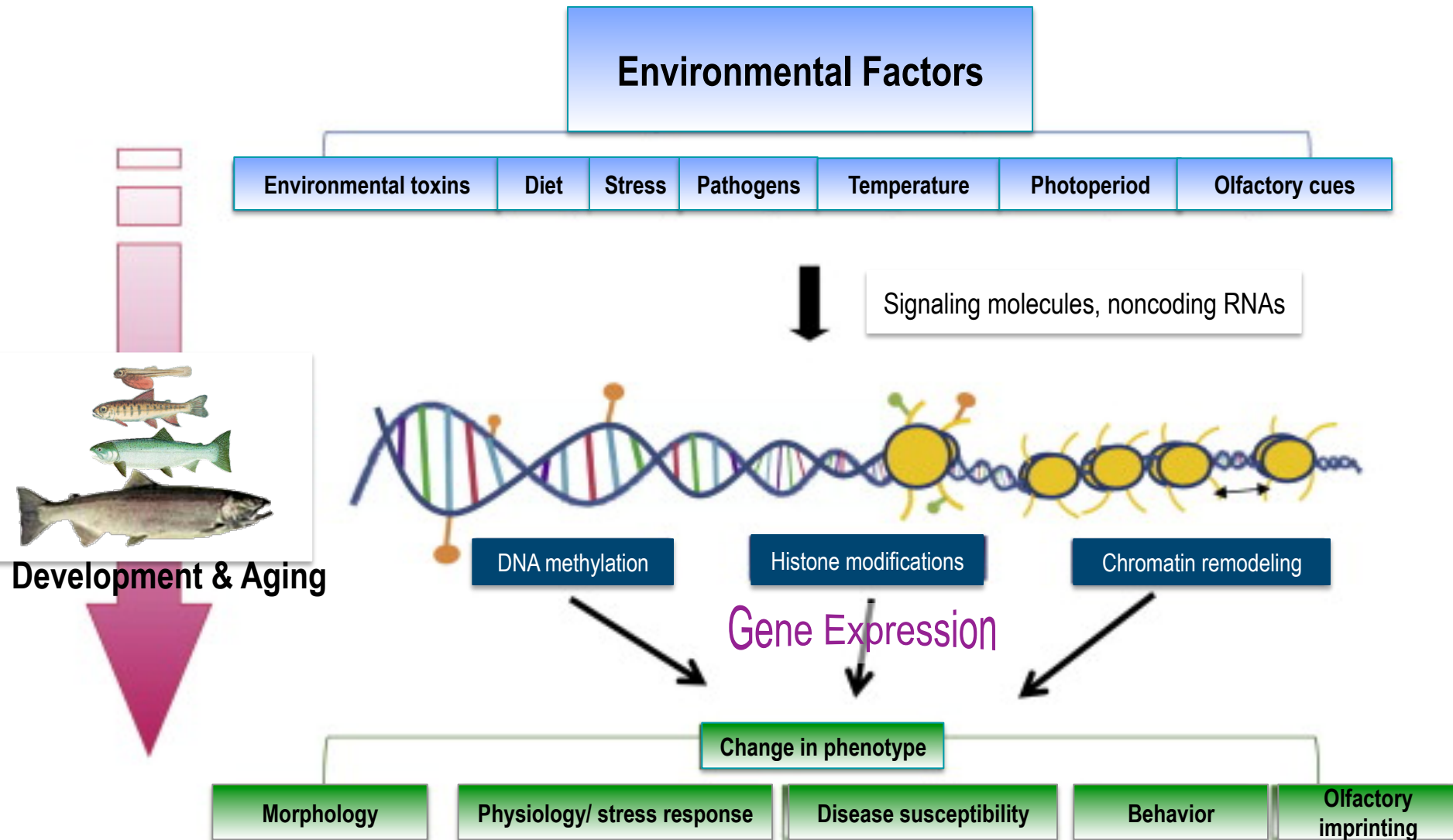
- 15,000 SNPs analyzed
- No obvious discrimination between hatchery and natural fish
- No association with brood year/age, rearing environment, or sex

Example of gene with DMR:

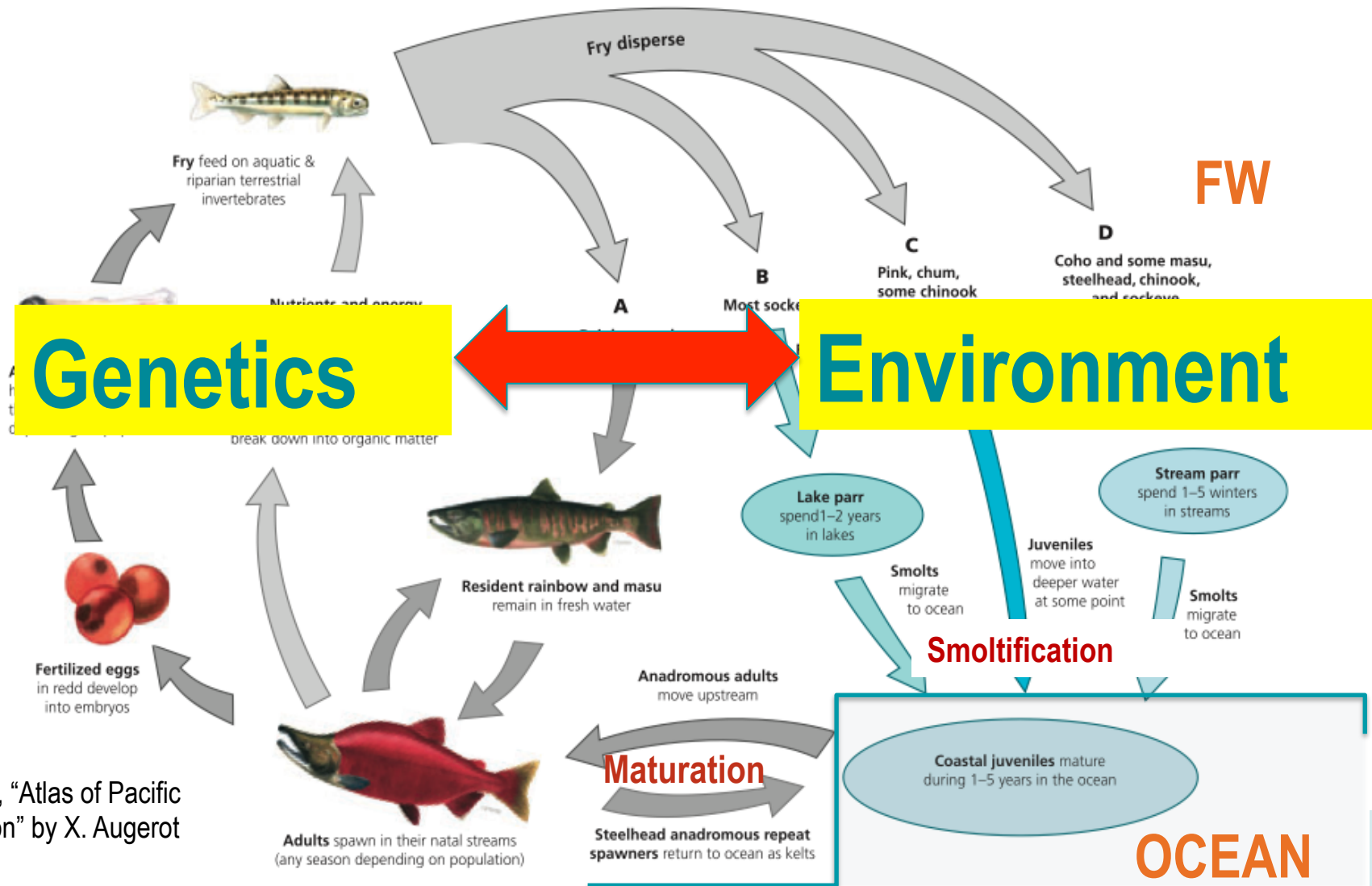
HTRA1, regulates insulin like growth factor (IGF) availability,
cleaves IGF binding proteins



Epigenetic Mechanisms: Link Between Nature and Nurture



Plasticity of Pacific Salmon and Steelhead Life Cycles



From, "Atlas of Pacific Salmon" by X. Augerot